Characterization of genes involved in the host specificity of *Salmonella enterica* serovars

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BACKGROUND

Typhoid fever is a disease specific to humans caused by infection with the bacteria *Salmonella enterica* serovar Typhi. Due to the strict host-specificity of the bacteria, the mouse models available to study typhoid fever are limited to humanized mice, which are expensive and time consuming to develop, or the use of the related bacterium *Salmonella enterica* serovar Typhimurium that results primarily in gastroenteritis. This study aims to characterize the function of select genes present in *S.* Typhimurium and absent in *S.* Typhi to better understand their involvement in host specificity. We generated a *S.* Typhimurium deletion mutant for the itaconate response operon (*S.* Typhimurium △*ripABC+lgl*) and used a competitive index infection model to determine whether the deletion of the *rip*ABC and *lgl* genes decrease the ability of the bacteria to replicate *in vivo* relative to the wildtype (WT) strain. The deletion mutant for *ripABC+lgl* had an impaired ability to replicate relative to the WT strain when co-infected with equal amounts. This finding was consistent in the gastrointestinal tract (cecum contents and feces) as well as in the systemic organs (spleen and liver). An *in vitro* assessment of growth in the presence of the antimicrobial compound itaconate, produced by host macrophages showed that the *S.* Typhimurium △*ripABC+lgl* was inhibited by itaconate. This suggests that in the murine host, these genes play an important role in itaconate resistance and confer greater overall fitness for the bacteria. By better understanding the function of genes implicated in *Salmonella* host-specificity, we hope to contribute to the development of a typhoid fever model through addition of genes such as *rip*ABC and *lgl*, combined with alterations in the host model to increase susceptibility to infection.

Hypothesis: The deletion of ***ripABC+lgl*** genes from ***S.*** Typhimurium will decrease their ability to infect and replicate within the mouse

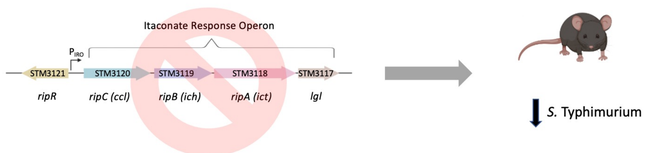


FIGURE 1. Mutant Construction

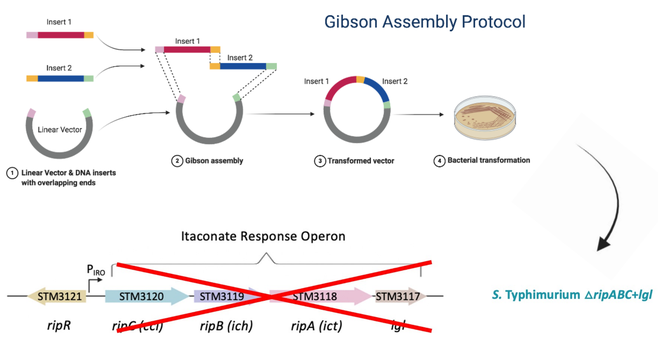
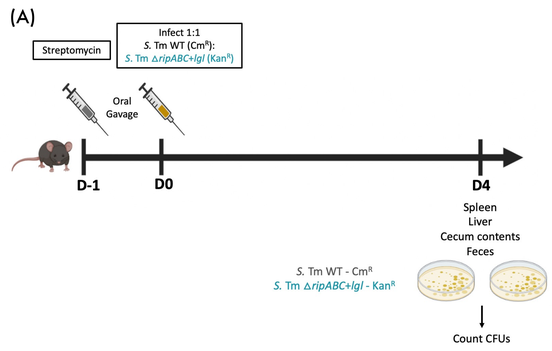


FIGURE 2. Decreased replication of *S.* Typhimurium △*ripABC+lgl* in the gastrointestinal tract and systemic organs of mice



(A) Timeline for competitive index infection of C57bl/6 mice. (B) Bacterial load in feces and (C) cecum following a competitive index infection. Mice were pre-treated with 20 mg of Streptomycin, then infected with a 1:1 ratio of *S.* Typhimurium WT (Cm^R) and *S.* Typhimurium mutant (Kan^R) via oral gavage for a total bacterial inoculation of $1x10^9$ CFU/mouse. Wildtype and mutant strains of *S.* Typhimurium were generated in a virulent and an avirulent background (△*invAspiB*) as a negative control to demonstrate conditions of low inflammation and low itaconate. (D) Bacterial load in the systemic organs of liver and spleen following a competitive index infection, as described in (B).

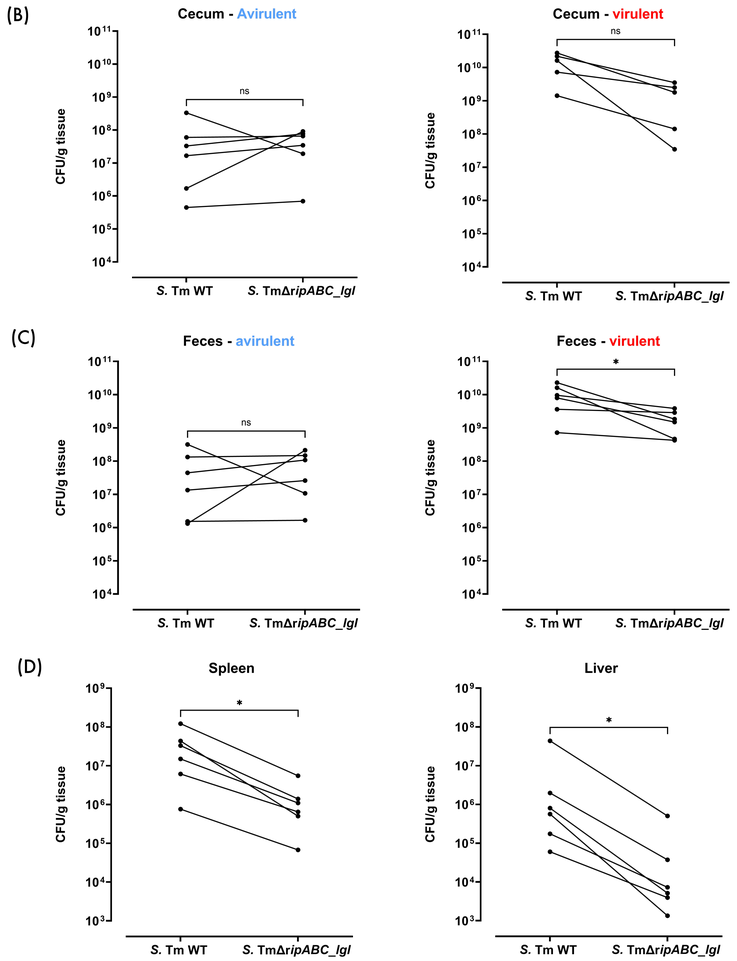
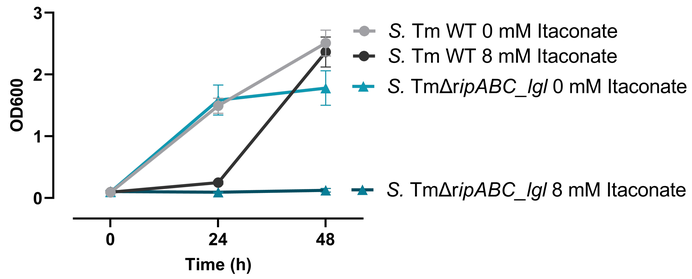


FIGURE 3. Itaconate inhibits the growth of *S.* Typhimurium △*ripABC+lgl in vitro*



Growth of *S.* Typhimurium WT and △*ripABC+lgl* incubated with or without itaconate in minimal media supplemented with acetate for 48 hours. *S.* Typhimurium WT growth is unaffected by itaconate while *S.* Typhimurium △*ripABC+lgl* growth is inhibited.

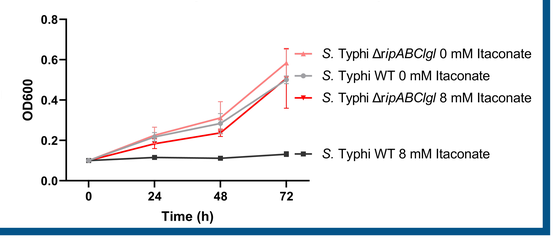
CONCLUSIONS

- *S.* Typhimurium △*ripABC+lgl* had decreased growth *in vivo* within the gastrointestinal tract and systemic organs when co-infected with *S.* Typhimurium WT in mice
- The growth of *S.* Typhimurium △*ripABC+lgl* was inhibited in the presence of physiological concentrations of itaconate *in vitro*

FUTURE DIRECTIONS

We aim to insert the *ripABC+lgl* genes into S. Typhi and infect mice to determine if there is an increased ability of the bacterial to replicate *in vivo*.

Preliminary Data



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